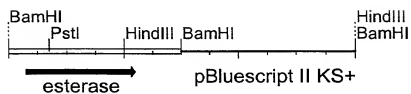
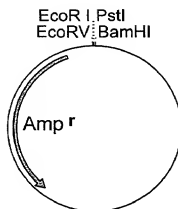


FIG. 1

pBMesterase1



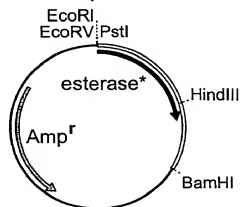
pBluescript II KS+



PstI/BamHI

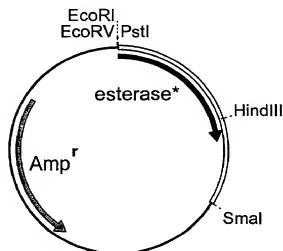
PstI/BamHI

Ligation



pBMesterase1a

Conversion of BamHI to SmaI



pBMesterase1b

FIG. 2

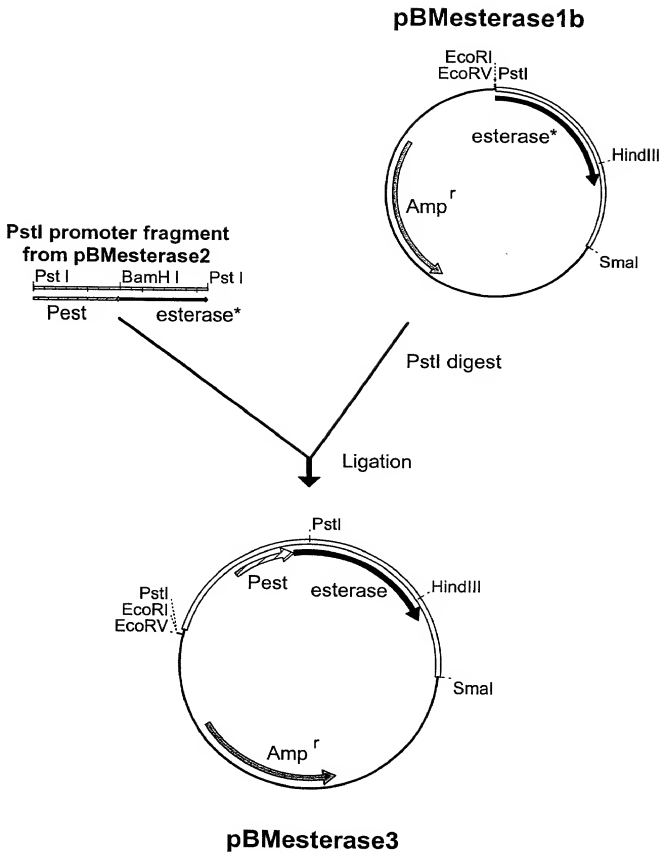
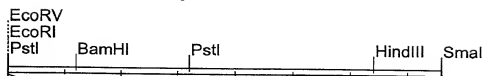


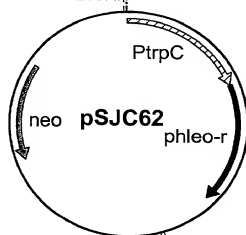
FIG. 3

SmaI/ EcoRV esterase fragment from pBMesterase3

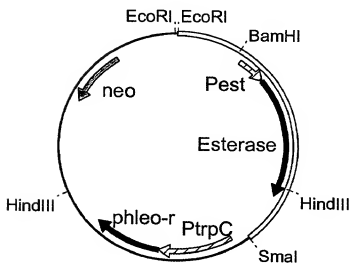


BamHI
SmaI
EcoRI

- 3.8 kb EcoRV/SmaI fragment inserted into SmaI site of pSJC62.



XbaI
HindIII



pSJC62.3

FIG. 4

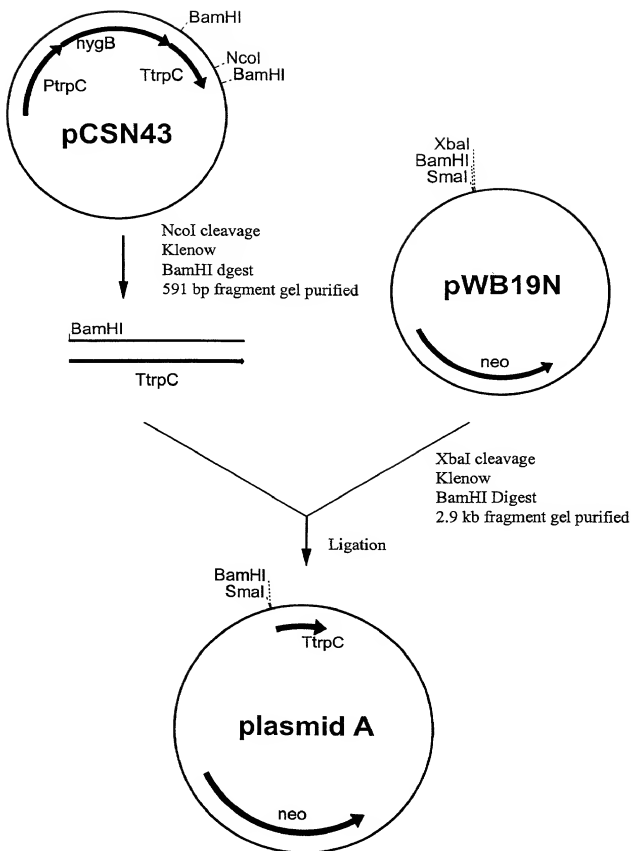


FIG. 5

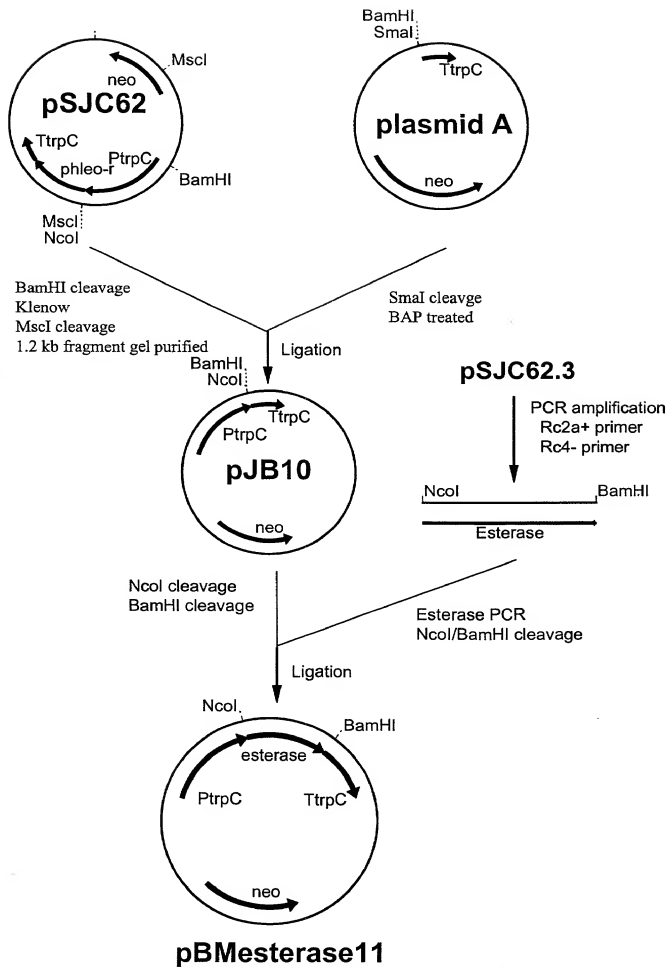


FIG. 6

N-TERMINAL AMINO ACID SEQUENCE

AMINO ACID SEQ.		T	N	P	N	E	P
REV. TRANSLATION		ACX	AAPy	CCX	AAPy	GAPu	CC
INVERSE		GGPy	TCPu	TTX	GGPu	TTX	GT
PROBE	1	GGPy	TCPu	TTG	GGPu	TTX	GT
	2			A			
	3			T			
	4			C			

Four 17-mer oligonucleotide probes each with a 32-fold degeneracy were synthesized from the N-terminal amino acid sequence and used to probe a Southern blot of *R.toruloides* DNA.

FIG. 7

RHODOSPORIDIUM ESTERASE cDNA

ATGCTCCTTAACCTCTTACCCCTCGCCTCCCTCGCTGCGACGCTCCAGCTCGCCTTTGGC 70
M L L N L F T L A S L A A T L Q L A F A

TCTCCGACCTCCCTCGTCCGCCGACGAACCCAAACGAGCCCCCTCCCGTCGTCGACCTC 130
S P T S L V R R T N P N E P P P V V D L

GGCTACGCCCGCTACCAAGGCTACTTGAACGAGACCGCCGACTCTACTGGTGGCGCGGA 190
G Y A R Y Q G Y L N E T A G L Y W W R G

ATCCGCTACGCCTCGGCTCAGCGCTTCCAGGCTCCTCAGACGCCCGCGACGCACAAGGCC 250
I R Y A S A Q R F Q A P Q T P A T H K A

GTCCGCAACGCGACTGAGTATGGACCGATCTGTTGGCCGGCTAGCGAGGGAACCAACCG 310
V R N A T E Y G P I C W P A S E G T N T

ACCAAGGGCTTGCCGCCGCTAGCAACAGCTCGAGCAGCGCCGCGCAGAAACAGGCGTCG 370
T K G L P P P S N S S S S A P Q K Q A S

GAGGATTGCCTCTTCTCAATGTCGTTGCCCGCCGGCTCGTGCAGGGCGACAATCTT 430
E D C L F L N V V A P A G S C E G D N L

CCCGTCCTCGTCTACATTACGGAGGTGGCTACGCCTTCGGCGATGCGAGCACC CGCAGC 490
P V L V Y I H G G G Y A F G D A S T G S

GACTTTGCCGCTTACCAAGCACACGGGAACCAAGATGGTGGTGTAAATCTCCAGTAC 550
D F A A F T K H T G T K M V V V N L Q Y

CGTCTCGGCAGCTTTGGTTTCTCGCTGGCCAAGCCATGAAGGACTACGGTGTAAACGAAC 610
R L G S F G F L A G Q A M K D Y G V T N

GCCGGCTTGCTTGACAGCAATTCGCCCTTCAATGGGTTCACAGCAGCTCTCGAAGTTC 670
A G L L D Q Q F A L Q W V Q Q H V S K F

GGCGCAACCCCCGATCACGTTACGATTTGGGGCGAGTCTCGAGCGCAGGGTCCGTTATG 730
G G N P D H V T I W G E S A G A G S V M

AACAGATCATTGCGAACGGCGGCAACCCGTCAAGGCTCTCGGTCTCAAGAAGCCCTC 790
N Q I I A N G G N T V K A L G L K K P L

TTCCAGCTGCCATCGGCTCCTCCGTCTTCTCCCTTACCAAGCCAAGTACAACCTCCCC 850
F H A A I G S S V F L P Y Q A K Y N S P

TTCCGCGAGCTGCTCTACTCCCAACTCGTCTCGGCGCAAACTGCACCAAGCCGCGCTCG 910
F A E L L Y S Q L V S A T N C T K A A S

TCCTTCGCTTGCCCTCGAAGCTGTGACGCTGCGGCGCTCGCTCGCGGCGGTGAAGAAC 970
S F A C L E A V D A A A L A A G V K N

TCGGCGGCGTTCCCGTTGGGTTTTGGTCGTATGTCCCGGTGCTGACGGGACCTTCTTG 1030
S A A F P F G F W S Y V P V V D G T F L

FIG. 8A

ACTGAGCGCGCTCGCTCCTTCTCGCCAAGGGCAAGAAGAACCTCAATGGCAACCTCTTC	1090
T E R A S L L L A K G K K N L N G N L F	
ACCGGGATCAACAACCTCGACGAAGGATTATATTCACTGACGCCACTATTGAGAAGAC	1150
T G I N N L D E G F I F T D A T I Q N D	
ACGATCAGCGACCACTCGCAGCGCGTCTCCAGTTCGACCGCCTCCTCGCGGCTCTTC	1210
T I S D Q S Q R V S Q F D R L L A G L F	
CCCTACATCACCTCGGAGGAGCGCCAGGCCGTCGCGAAGCAGTACCCGATCTCCGACGCG	1270
P Y I T S E E R Q A V A K Q Y P I S D A	
CCGTCAAAGGGCAACACCTTCTCTCGCATCTCGGCCGTCATCGCGGACTCGACCTTCGTC	1330
P S K G N T F S R I S A V I A D S T F V	
TGCCCGACCTACTGGACCGCCGAGGCGTTCGGCTCGTCCGCCACAAGGGCTCTTCGAC	1390
C P T Y W T A E A F G S S A H K G L F D	
TACGCGCGGCTCACCACGCGACCGACAACCTCGTACTACATCGGCTCCATCTGGAACGGC	1450
Y A P A H H A T D N S Y Y I G S I W N G	
AAGAAGTCGGTCTCGTCCGTCCAGTCCTTCGACGGCGCGCTCGGCGGCTTCATCGAGACG	1510
K K S V S S V Q S F D G A L G G F I E T	
TTCAACCCGAACAACACGCTGCCAACAAGACCATCAACCCCTTACTGGCCGACGTTTCGAC	1570
F N P N N N A A N K T I N P Y W P T F D	
TGGGCAAGCAGCTCCTCTTCAACACGACGACGAGGGACACCTCTCTCCCGCCGACCCG	1630
S G K Q L L F N T T T R D T S P A D P	
CGCATCGTTGAGACTTCAAGCTTGACCGACTTTGGCAGGAGCCAGAAGACCAAGTGCAC	1690
R I V E T S S L T D F G T S Q K T K C D	
TTCTGGCGTGGGTCAATCTCGGTGAACGCGGTCTC	1726
F W R G S I S V N A G L	

FIG. 8B

GGATCCACCCGAACCTCTGTCCCGCTTTCTGGCTTTCTTCCTTGCTGTGCCCCCATCGCCT 60

|-- Translation Start -->

TTCCCCGACTCGCGCCGCATGCTCCTTAACCTCTTCACCCCTCGCCTCCCTCGCTGCGACGCT 120
M L L N L F T L A S L A A T L

CCAGCTCGCCTTTGGCTCTCCGACCTCCCTCGTCCGCGGCACGAACCCAAACGAGCCCCC 180
Q L A F A S P T S L V R R T N P N E P P

TCCCGTCGTGACCTCGGTACGCCCGCTACCAAGGCTACTTGAACGAGACCGCGGACT 240
P V V D L G Y A R Y Q G Y L N E T A G L

CTACTGGTGGCGCGGAATCCGCTACGCCTCGGCTCAGCGCTTCCAGGCTCCTCAGACGCC 300
Y W W R G I R Y A S A Q R F Q A P Q T P

CGCGACGCACAAGGCCGTCCGCAACGCGACTGAGTATGGACCGATCTGTTGGCCGGCTAG 360
A T H K A V R N A T E Y G P I C W P A S

CGAGGGAACCAACACGACCAAGGGCTTGGCGCCGCTAGCAACAGCTCGAGCAGCGCGCC 420
E G T N T T K G L P P P S N S S S S A P

GCAGAAACAGGCGCTCGGAGGATTGCCTCTTCTCAATGTCGTTGCCCCCGCGGGCTCGTG 480
Q K Q A S E D C L F L N V V A P A G S C

CGAGGGCGACAATCTTCCCGCTCTGCTACATTACGGAGGTGGCTACGCTTCCGCGGA 540
E G D N L P V L V Y I H G G G Y A F G D

TGCGAGCACCGGCGAGCGACTTTGCCCGCTTACCAAGCACACGGGAACCAAGATGGTCGT 600
A S T G S D F A A F T K H T G T K M V V

TGTAATCTCCAGTACCGTCTCGGCAGCTTTGGTTTCCTCGCTGGCCAAGCCATGAAGGA 660
V N L Q Y R L G S F G F L A G Q A M K D

[---- Intron #1 ----]

CTACGGTGTAAACGAACCGCGGCTTGCTTGACCAGGTGAGTTTCCCGCATGATACCGCCC 720
Y G V T N A G L L D Q

-----]

ACCTTTCGACTCATGCTGACGCCTCTCCCGCTCGCAGCAATTGCGCCCTTCAATGGGTTCA 780
Q F A L Q W V Q

ACAGCACGTCTCGAAGTTCGGCGGCAACCCCGATCACGTTACGATTGGGGCGAGTCTGC 840
Q H V S K F G G N P D H V T I W G E S A

[---- Intron #2 ----]

AGGGCGAGGGTCCGTTATGAACCAGATCATTGCGAACGTGAGCCACCCGAACCGATCTCC 900
G A G S V M N Q I I A N

-----]

AGCGGACTTTCCCCCCCCCCCCCGCTGACCTCCCTCGTCTTGCAGGGCGGCAACA 960
G G N T

CCGTCAGAGGCTCTCGGTCTCAAGAAGCCCTCTTCCACGCTGCCATCGGCTCCTCCGTCT 1020
V K A L G L K K P L F H A A I G S S V F

TCCTCCCTTACCAAGCCAAGTACAACCTCCCGCTTCGCGGAGCTGCTCTACTCCCAACTCG 1080
L P Y Q A K Y N S P F A E L L Y S Q L V

FIG. 9A

CTGCGGCGCTCGCTGCGGCGGGCGTGAAGAACTCGGCGGCGTTCGCGTTCGGGTTTGGT 1200
A A L A A A G V K N S A A F P F G F W S

CGTATGTCCCGGTCGTCGACGGGACCTTCTTGACTGAGCGCGCGTCGCTCCTTCTCGCCA 1260
Y V P V V D G T F L T E R A S L L L A K

[---- Intron #3 -----]
AGGGCAAGAAGAACCTCAATGGCGTGGCTGCGAGCTTTCGAGTGCTTCAGGATCTCGCT 1320
G K K N L N G

-----]
GACACTGTCGACCGGCTCGCAGAACCTCTTCACCGGGATCAACAACCTCGACGAAGATGA 1380
N L F T G I N N L D E G

---- Intron #4 -----]
GTTCCCGTCGACGGCTCTGTTTCGCCCAGCGAGACTGACTTGTCTTTTCGAAGATTACG 1440

ATTATATTCACTGACGCCACTATTGAGAAGACACGATCAGCGACCGAGTCGACGCGCT 1500
F I F T D A T I Q N D T I S D Q S Q R V

CTCCAGTTCGACCGCTCTTCGCGGCTCTTCCCTTACATCACCTCGGAGGAGCGCCA 1560
S Q F D R L L A G L F P Y I T S E E R Q

GGCCGTCGCGAAGCAGTACCCGATCTCCGACGCGCCGTCAAAGGGCAACACCTTCTCTCG 1620
A V A K Q Y P I S D A P S K G N T F S R

[---- Intron #5 -----]
CATCTCGGCGTCATCGCGGACTCGACCTTCGTGTGCGTTCCTCCGTCGCTTCTCCGAGT 1680
I S A V I A D S T F V

-----]
ATTCCGCTGACTTCCCGCTTGCCCGCAGCTGCCCGACCTACTGGACCGCGAGGCGTTG 1740
C P T Y W T A E A F G

GCTCGTCCGCCCCACAAGGCCTCTTCGACTACGCGCGGCTCACACGCGACCGACAAC 1800
S S A H K G L F D Y A P A H H A T D N S

CGTACTACATCGGCTCCATCTGGAACGGCAAGAAGTCGGTCTCGTCCGTCAGTCTCTCG 1860
Y Y I G S I W N G K K S V S S V Q S F D

ACGGCGCGCTCGGCGGCTTCATCGAGACGTTCAACCCGAACAACAGCTGCCAACAGA 1920
G A L G G F I E T F N P N N N A A N K T

CCATCAACCTTACTGGCGGACGTTTCGACTCGGGCAAGCAGCTCCTCTTCAACACGACGA 1980
I N P Y W P T F D S G K Q L L F N T T T

CGAGGACACCTCTCTCCCGCGGACCGCGCATCGTTGAGACTTCAAGCTTGACCGACT 2040
R D T L S P A D P R I V E T S S L T D F

TTGGCAGGACCAAGAAGCAAGTGCAGCTTCTGGCGTGGGTCAATCTCGGTGAACGCGG 2100
G T S Q K T K C D F W R G S I S V N A G

GTCTCTAGGCGCTTTCTCTCCGACTTCCTTCGTTCTTTTCGTTGTTTATTCTGCAGTTC 2160
L *

CGTTGTATCGGCATTCTGTCGCTGTAGCTCACTCGAGTATAGACGTTGGCAAGTGCAGAA 2220

FIG. 9B

TRN 2-1738 RHODOSPORIDIUM ESTERASE cDNAs

┌Translation Start ┌Mature Peptide
MLLNLF¹TLASLAATLQLAFASPTSLVRR²TNPNEPPPVVDLG³YARYQGYLNETAGLYWWRG
IRYAS⁴AQRFQAPQTPATHKAVRNATEYGPICWPASEGNTTTKGLPPPSN⁵SSSSAPQKQAS
EDCLFLNVVAPAGSCEGDNLPVLVYIHGGGYAFGDASTGSDFAAFTKHTGTKMVVVNLQY
RLGSF⁶GFLAGQAMKDYGV⁷TNAGLLDQQFALQWVQ⁸QHVS⁹KFGGNPDHVTIWGESAGAGSVM
NQIIANGGNTVKALGLKKPLFHAAIGSSVFLPYQAKYNSPFAELLYSQLVSATNCTKAAS
SFACLEAVDAAALAAAGVKNSAAFFPGFWSYVPVVDGTFILTERASLLAKGKKNLNGNLF
TGINNLD¹⁰EGFI¹¹FTDATIQNDTISDQSQRV¹²SQFDRLLAGLFFYITSEERQAVAKQYPI¹³SDA
PSKGNTFSRISAVIADSTFVCPTYWTA¹⁴EAFGSSAHKGLFDYAPAHHATDNSYYIGSIWNG
KKS¹⁵VSSVQSFDGALGGFIETFPNNNAANKTINPYWPTFDSGKQLLENTTT¹⁶RTLSPADP
RIVETSSLTDFGTSQKTKCDFWRGSISVNAGL*

FIG. 10

Amino acid composition from 1 to 572
 TRN 2-1738 RHODOSPORIDIUM ESTERASE cDNA

	Total	Percent
A	67	11.7
C	7	1.2
D	25	4.4
E	16	2.8
F	35	6.1
G	49	8.6
H	9	1.6
I	21	3.7
K	25	4.4
L	48	8.4
M	4	0.7
N	35	6.1
P	31	5.4
Q	26	4.5
R	16	2.8
S	52	9.1
T	43	7.5
V	32	5.6
W	10	1.7
Y	21	3.7
Acidic	41	7.2
Basic	41	7.2
Charged	82	14.3
Net charge	0	0.0
Hydrophobic	136	23.8
Residues	572	
MW	61334	

FIG. 11